MODULATION OF THE HOST TRANSCRITOME BY *COXIELLA BURNETII* NUCLEAR EFFECTORS

An Undergraduate Research Scholars Thesis

by

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Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Research Advisor: Dr. James Samuel

May 2014

Major: Biology

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ABSTRACT

Modulation of the Host Transcriptome by *Coxiella* burnetii Nuclear Effectors. (May 2014)

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Coxiella burnetii is an infectious bacterium that causes Q-fever in mammals. Central to pathogenesis is a Dot/Icm Type IV secretion system (T4SS) which is similar to the secretion system of other pathogens. The proteins secreted by the T4SS are essential for modulating certain cellular processes such as apoptosis, intracellular replication and vacuole formation. It was hypothesized that these proteins accomplished these functions by binding to the host cell's nuclear genome. Chromatin Immunoprecipitation (ChIP) was used to determine which nuclear T4SS proteins bound to host DNA. The immunoprecipitated DNA was sequenced to determine what genes were present and their function within the host cell. Sequencing revealed that Cbu1314 bound host genes associated with important cell functions such as cell-cell signaling, apoptosis, and regulation of the cell cycle. The modification of these genes could lead to an incomplete host response and allow the continued replication of *C. burnetii*. These results suggest that the nuclear effector Cbu1314 binds host DNA and may modulate the host response by manipulation of host chromatin.

ACKNOWLEDGEMENTS

I would like to thank Dr. James Samuel for giving me the opportunity to work in a lab and pursue my goal of undergraduate research. I would like to thank Mary Weber, for taking me under her wing and for always being there whenever I needed help. I would like to thank Kristina Rowin, Erin van Schaik, Robert Faris, Juanita McLachlan, Smita Singh and Chris Dealing for answering whatever questions I had and helping me to correct my mistakes. Finally, I wanted to thank William Wright, Mallory Brown, Caitlyn Cowen and Jennifer Parra for making sure I always had what I needed to get the job done.

NOMENCLATURE

GFP	Green Fluorescent Protein
EGFP	Enhanced Green Fluorescent Protein
ChIP	Chromatin Immunoprecipitation
T4SS	Type 4 Secretion System
Dot/Icm	Defect in organelle trafficking/intracellular multiplication

CHAPTER I

INTRODUCTION

Background

Coxiella burnetii is a Gram negative intracellular pathogen that causes acute and chronic Q fever in humans (1). This pathogen is very infectious due to its ability to spread via aerosols and inherit resistance to environmental stressors (1). This organism possesses a Dot/Icm T4SS that is closely related to the system of *Legionella pneumophila*. This system is used to translocate bacterial virulence proteins, called effectors, in to the host cell's cytoplasm. A functional T4SS is required for vacuole formation, intracellular replication, effector translocation and modulation of apoptosis (1).

Following release into the host cell cytosol, many secreted effector proteins traffic to distinct subcellular compartments. By expressing a large collection of *C. burnetii* T4SS substrates as C-terminal fusions to EGFP, we have identified 6 effectors that traffic to the nucleus of the host cell (1). Other pathogens such as *Legionella*, *Chlamydia* and *Anaplasma*, secrete nuclear effectors that bind to chromatin and modulate the host response to the invading pathogen (2, 3). Given that this is a conserved general strategy among nuclear effectors from intracellular pathogens, we speculate that a subset of *C. burnetii* localized nuclear effectors modulate the host response by direct binding to chromatin or by targeting other eukaryotic proteins associated with host chromatin.

Hypothesis

We hypothesize that one of these nuclear effectors, Cbu1314, influences host response to infection by manipulating the host transcriptome.

CHAPTER II

METHODS

A ChIP-Seq protocol determined if the nuclear effectors of *C. burnetii* bound to DNA (2). HEK293 cells were transfected with EGFP-fused effectors or the vector alone as a negative control. After 24 hours, cells were lysed using the Active Motif ChIP-It express kit (Active Motif). The effector proteins and the bound DNA will be purified from the rest of the solution using protein G beads and anti-GFP antibody. Proteinase K degraded the effector proteins and the remaining bound DNA was purified using the Active Motif immunoprecipitation kit. The DNA was sent to Active Motif for deep sequencing. If the sequencing indicated that a nuclear effector bound to DNA, aliquots of the effector-transfected HEK293 cells were taken representing cytoplasmic, soluble nuclear and chromatin fractions. These three fractions were analyzed using Western immunoblotting and acted as a second confirmation that the nuclear effector was bound to host cell chromatin.

The sequencing of the chromatin that was found bound to a nuclear effector was analyzed for motifs using Multiple EM for Motif Elicitation (MEME). This program found the most common motifs present within the effector-bound chromatin and allow for further analysis of the genes within the DNA sequence. The sequenced DNA from Active Motif provided a long list of genes along with their molecular and biological function, as well as the host cell component that they were associated with. The most common functions and cell components of each of these categories was identified and grouped together in order to further analyze what kind of genes were present in the effector-bound chromatin.

Characterization of these nuclear effectors was essential to further our understanding of host-pathogen interactions and may lead to novel vaccine candidate targets.

CHAPTER III

RESULTS

Protein Cbu1314 was detected in HEK293 host cells that had been transfected with Flag-tagged Cbu1314. As shown in Figure 1, Cbu1314 localized to all three fractions taken. These results suggest that this effector might bind host DNA.

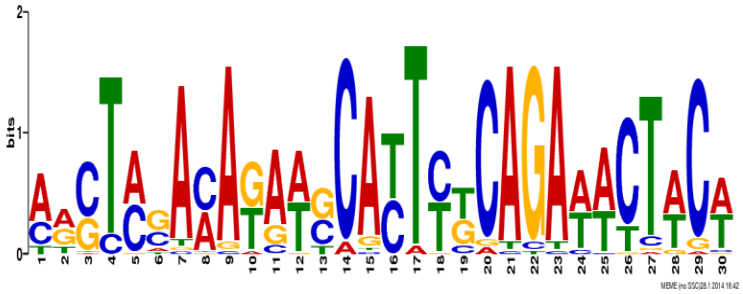
1 

Figure 1: **Ectopically expressed Cbu1314 associates with host chromatin**. HEK293 cells were transiently transfected with Flag-tagged Cbu1314 and the cytoplasmic (1), soluble nuclear (2), and chromatin (3) fractions were isolated using Thermo chromatin isolation kit. Data are representative of at least 3 independent experiments.

To determine what genomic regions Cbu1314 binds, transfected HEK293 cells were sent to active motif for ChIP-seq analysis. Reads were generated on the Illumina 454 and resulted in over 7 million reads, which mapped to over 850 genes. The resulting intervals were analyzed using MEME to determine what motifs were present. Motif1 (Fig 2A), Motif2 (Fig 2B) and Motif3 (Fig 2C) were the most prevalent motifs observed from the sequenced genes. 61 (7.8%) of all the genes had Motif1, 41 (5.3%) had Motif2 and 67 (8.6%) had Motif3. All 3 motifs were mostly AT rich.

2A.

2B.



2C.

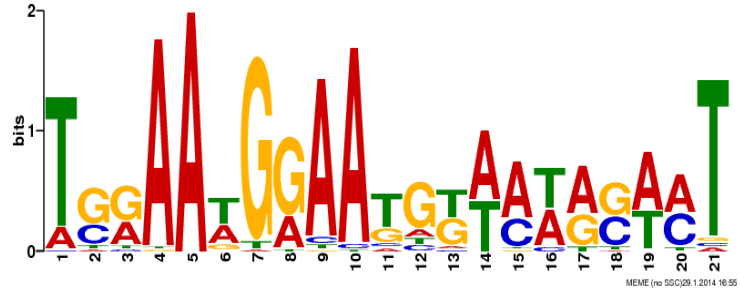
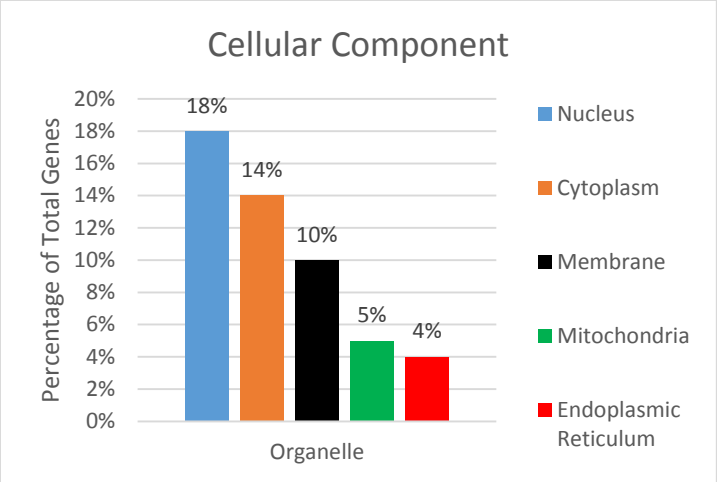
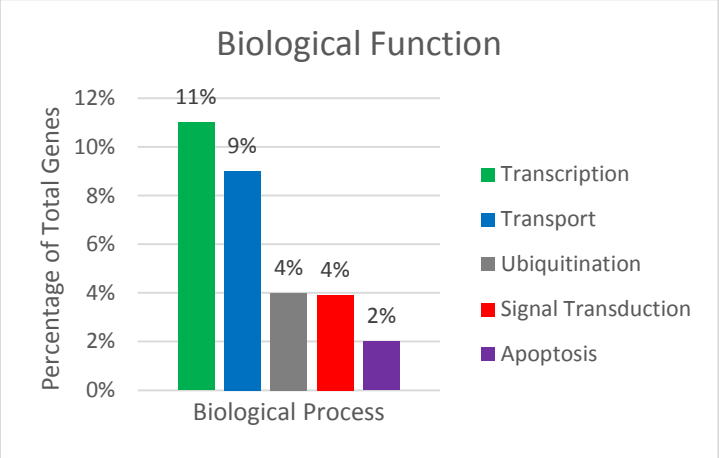


Figure 2: **Cbu1314 binds to AT-rich genomic regions .** To determine regions bound by the nuclear effector, Cbu1314, ChIP-seq was conducted and the isolated DNA was sequenced using the Illumina 454. Analysis of the DNA sequences identified 3 AT-rich motifs (B-D).

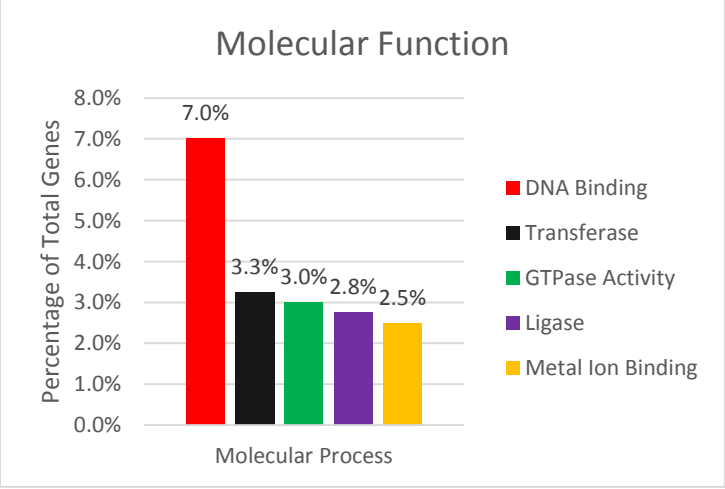
The sequenced genes were individually analyzed based on their biological and molecular function, as well the host cellular component that was associated with it.


Cellular Component
Percentage of Total Genes
20%
18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
18%
14%
10%
5%
4%
Organelle
Nucleus
Cytoplasm
Membrane
Mitochondria
Endoplasmic Reticulum

3A


Biological Function
Percentage of Total Genes
12%
10%
8%
6%
4%
2%
0%
11%
9%
4%
4%
2%
Biological Process
Transcription
Transport
Ubiquitination
Signal Transduction
Apoptosis

3B


Molecular Function
Percentage of Total Genes
8.0%
7.0%
6.0%
5.0%
4.0%
3.0%
2.0%
1.0%
0.0%
7.0%
3.3%
3.0%
2.8%
2.5%
Molecular Process
DNA Binding
Transferase
GTPase Activity
Ligase
Metal Ion Binding

3C

Figure 3

The sequenced genes were organized by their biological function and molecular function, as well as the associated cellular component. Each of the three graphs show the 5 most common functions or components for each category. Almost 20% of the sequenced genes were found to affect the nucleus (Fig 3A). Just over 10% of the biological function of all genes involved transcription (Fig 3B). The molecular function of the genes was more balanced out, with DNA Binding accounting for around 7% (Fig 3C).

CHAPTER IV

DISCUSSION

We predicted that certain nuclear effectors would modulate host response by binding to certain regions within the host nucleus. Cbu1314 was found to be attached to host chromatin by the ChIP-Seq experiment, and sequencing of this host chromatin led to the identification of a large amount of host genes. The majority of these genes were involved in various biological and molecular processes that are associated with the host nucleus and with host response against an infection. In conclusion:

- Cbu1314 is a nuclear effector protein that is secreted by the T4SS of *C. burnetii*
- Cbu1314 binds to host genes associated with transcription, ubiquitination and apoptosis
- Cbu1314 potentially binds to 3 motifs in the host genome
- *C. burnetii* and its nuclear effector Cbu1314 may modulate the host transcriptome

For future experiments with Cbu1314, the chromatin from the host nucleus can be isolated and run with purified Cbu1314 using gel-shift assays in order to further confirm that the protein binds to host-DNA sequence. Gel-shift assays using biotylinated oligos would determine if binding is specific to the identified motifs. In order to determine whether Cbu1314 modifies expression of these genes, RT-PCR will be used to determine if these genes are differentially expressed under transfection or infection conditions

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